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SHORT COMMUNICATION

Resveratrol Reduces Oxidative Stress and Improves Arsenic Efflux in Rats Exposed To Arsenic Trioxide

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ABSTRACT

The effects of resveratrol on arsenic trioxide-induced oxidative stress and arsenic efflux in spleen were determined in Wistar rats exposed to arsenic trioxide (As_2O_3). Wistar rats were segregated into four groups: control (0.9% normal saline), As_2O_3-treated (2 mg/kg for 7 days, i.p.), resveratrol+As_2O_3-treated, resveratrol-treated (5 mg/kg for 7 days, i.p.). Spleen was analyzed for lipid peroxidation, antioxidant enzyme activities, glutathione redox system and arsenic accumulation. Arsenic treatment showed significant increases in the malondialdehyde, reactive oxygen species and arsenic accumulation in spleen. Furthermore, arsenic treatment decreased significantly activity of superoxide dismutase and total glutathione/ oxidized glutathione ratio. However, treatment with resveratrol to arsenic-treated rats reduced oxidative stress and improved arsenic efflux. These findings may provide the potential strategy for arsenic-induced spleen toxicity and As_2O_3 as a single agent causing toxic effects.

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INTRODUCTION

Arsenic is a naturally occurring, toxic substance that exists ubiquitously in the soil, food and water, and causes a wide range of system toxic effects to human and animals, such as cancer, heart disease and diabetes (Sharaf *et al*., 2013; Khan *et al*., 2013). Many literatures reported that toxicity of arsenic is mediated by the generation of reactive oxygen species (ROS) (Bolt, 2013). ROS accumulation can result in obvious damage to cell structures, which aggregates into a situation called oxidative stress (Flora, 2011). However, one therapy for preventing or attenuating the generation of free radicals during arsenic exposure is the use of antioxidants. The recent study revealed that antioxidants can preserve or decrease arsenic exposure induced oxidative stress (Nandi *et al*., 2005).

Resveratrol (trans-3, 4, 5-trihydroxystilbene) is from a variety of medicinal plants, grape, and peanut etc. It has been reported to have anti-inflammatory, antioxidant, and anti-carcinogenic effects (Yu *et al*., 2013). Karabulut *et al*. (2006) demonstrated that there were protective effects of resveratrol on spleen in rats subjected to ischemia-reperfusion. On the basis of multitargeted agent of resveratrol, this study was conducted to evaluate the effects of treatment with or without resveratrol on As_2O_3-induced oxidative stress and arsenic efflux in spleen.

MATERIALS AND METHODS

Animals and treatments: Male Wistar rats, weighing 160-180 g, were purchased from the Experimental Animal Centre of Harbin Medical University (Harbin, China), strictly with the guidelines of the institutional ethics committee, and were housed under the conditions (22±2°C, 50±20% relative humidity) with rat diet and drinking water *ad libitum*. After acclimatizing for one week, 32 rats were divided into four equal groups: the control (0.9% normal saline), As_2O_3-treated (2 mg/kg for 7 days, i.p.), resveratrol+As_2O_3-treated (As_2O_3: 2 mg/kg; resveratrol: 5 mg/kg for 7 days, i.p.), resverarol-treated (5 mg/kg for 7 days, i.p.) group. The dose of resveratrol (5 mg/kg/d, i.p.) was selected from our recent studies (Zhang *et al*., 2013), while As_2O_3 (2 mg/kg/d, i.p.) was based on IPR-RAT LD_{50} values (13 mg/kg). On the 8th day, rats

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were given ether anesthesia and sacrificed according to approval of Ethics Committee/Animal care committee. Spleen tissues were quickly removed and homogenized in neutral phosphate-buffered saline using an Ultrathurax T25 Homogenisator, then centrifuged at 10,000×g for 10 min at 4°C.

Determination of parameters associated with oxidative stress and arsenic efflux: The activities of superoxide dismutase (SOD), reactive oxygen species (ROS), malondialdehyde (MDA) and total glutathione/oxidized glutathione ratio (T-GSH/GSSG) in spleen were determined according to manufacturer's instructions (Jiancheng Bioengineering Institute, China). Arsenic contents in the spleen tissues of all rats were wet-digested following the perchloric acid–nitric acid method. Arsenic concentration was determined using a Beijing Jitian Instrument Co., AFS930 atomic fluorescence spectrometry system (Beijing, China) as described in our previous study (Zhang *et al*., 2013).

Statistical analysis: The data were expressed as the means±standard errors. Statistical calculations were performed using SPSS v. 16.0 (SPSS Inc, Chicago, IL, USA). The differences between groups were examined by variance analysis. In all cases, $P<0.05$ represents a significant difference.

RESULTS AND DISCUSSION

The structure of the spleen is the connection with cellular immune function in rat. Arsenic exposed population is susceptible to opportunistic infections like tuberculosis and parasitic infections (Soto-Peña *et al*., 2006). Thus, it is extremely urgent to explore a substance preventing or decreasing arsenic-induced spleen toxicity. Several studies about arsenic toxicity were closely associated with an increase in the formation of ROS (Flora, 2011; Bolt, 2013). In our study, rats treated with As_2O_3 were observed higher MDA and ROS contents in spleen in Table 1. Resveratrol, a potent antioxidant and a putative activator of *Sirtuin* 1, is a phytoalexin present in at least 72 plant species, which are consumed by humans, such as mulberries, peanuts, and grapes (Yu *et al*., 2013). However, the presence of resveratrol attenuated MDA and ROS levels in spleen by 25.2 and 24.8%, respectively ($P<0.05$).

In the normal cell, SOD and glutathione play crucial roles as cellular antioxidants (Yu *et al*., 2013). As shown Table 1, significant decrease in antioxidant activities of SOD and T-GSH/GSSG ratio in spleen were also observed in rats treated with As_2O_3 ($P<0.05$). However, treatment with resveratrol preserved antioxidant enzymes activities. Therefore, we suggest the presence of resveratrol attenuated As_2O_3-induced oxidative stress by decreasing levels of MDA, ROS production and preserving levels of SOD, T-GSH/GSSG ratio, thereby protected antioxidant capacity in spleen, which may be the relation to the antioxidant activity of resveratrol, scavenging (neutralizing) free radicals, and maybe prevent depletion of levels of T-GSH and reduced GSH.

To evaluate whether resveratrol affects arsenic efflux in spleen, arsenic concentration in spleen was performed. Compared with the control, there were higher ($P<0.05$) in arsenic concentration of spleen in As_2O_3 group. Similarly, treatment with resveratrol reduced markedly the total arsenic accumulation in spleen ($P<0.05$), whereas resveratrol alone had no effect on arsenic accumulation. Consequently, the presence of resveratrol reduced arsenic burden in spleen by facilitating arsenic efflux.

Many reports in the literature that null genotypes of arsenic (III) methyltransferase, metallothionein, multidrug-resistance mdr1a/1b and glutathione S-transferase π are susceptible to suffer from arsenic toxicity (Zhang *et al*., 2013). Taken together, resveratrol may attenuate arsenic accumulation in spleen under a multiple regulatory mechanism, such as up-regulating arsenic efflux protein expression or down-regulating arsenic absorption protein expression. Duan *et al* (2013) demonstrated that resveratrol preserved splenic immunity of restraint stressed mice involved in autophagy. In our study, apart from ROS clearance and maintenance of antioxidant capacity, improving arsenic efflux is included as a potential mechanism. However, the further multi-mechanism is required to explore.

Table 1: Effect of As_2O_3 and resveratrol on antioxidative capacity and arsenic efflux

Parameters	Control	As_2O_3	As_2O_3+ Resveratrol	Resveratrol
MDA (n mol/mg)	1.59±0.13a	2.78±0.19b	2.08±0.15b	1.60±0.18a
ROS (U/mg)	27.85±2.64a	43.19±2.71b	32.48±2.43b	28.52±2.50a
SOD (U/mg)	88.62±8.09a	65.06±5.97b	80.69±5.39b	87.69±6.67a
T-GSH/GSSG	4.01±0.25a	1.65±0.2b	3.52±0.19b	3.91±0.25a
Total arsenic (μg/g)	1.64±0.12a	3.35±0.12b	2.63±0.11b	1.62±0.13a

Values (mean±SE) is a row bearing different alphabets differ significantly ($P<0.05$) than control group. Malondialdehyde (MDA), reactive oxygen species (ROS), superoxide dismutase (SOD), T-GSH/GSSG ratio, total glutathione/oxidized glutathione ratio.

Conclusion: This study illustrated that resveratrol treatment preserved spleen toxicity in rats exposure to As_2O_3. It is meaningful to find that resveratrol protected against arsenic exposure via which reduced oxidative stress and improved arsenic efflux.

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